

USDA/APHIS
Draft Environmental Assessment

In response to Permit applications 06-278-01r, 06-278-02r and 06-285-02r
received from Ventria Bioscience
To conduct field plantings of rice (*Oryza sativa*) genetically engineered to
express human lysozyme, lactoferrin and serum albumin

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services

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I. Summary

The U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service, Biotechnology Regulatory Services (APHIS/BRS) has prepared an environmental assessment (EA) in response to permit applications (APHIS numbers 06-278-01r, 06-278-02r and 06-285-02r) received from Ventria Bioscience, Sacramento, California, to grow genetically engineered rice (*Oryza sativa* L.) in Geary County, Kansas. These transgenic plants have been modified to express the human (*Homo sapiens*) proteins lysozyme, lactoferrin or serum albumin. The plants expressing lysozyme and lactoferrin have also been engineered with the selectable marker gene *hpt*¹ which encodes for the enzyme hygromycin B phosphotransferase (Hpt). Hpt inactivates the antibiotic hygromycin. Plants containing serum albumin also contain either the *hpt* marker gene and/or the phosphinothricin acetyltransferase (*pat*) gene which allows laboratory selection of transformed tissues using bialaphos herbicide. None of the marker genes are expressed in mature plants due to the nature of the gene promoters.

This environmental assessment was prepared in accordance with: (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.C § 4321 et seq.); (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR §§ 1500-1508); (3) USDA regulations and implementing NEPA (7 CFR § 1b); and (4) APHIS NEPA Implementing Procedures (7 CFR § 372).

These plantings in Kansas should be harvested in the fall of 2007. Similar plantings are planned in future years.

The bases of confinement for these field tests are:

- The field test sites are located on private lands in Geary County, KS. There is no commercial rice grown in the State of Kansas.
- In nature, chromosomal genetic material of rice can only be transferred to other sexually compatible plants by cross-pollination. Rice is primarily self pollinating and outcrosses at a very low frequency. Furthermore, the only sexually compatible plants in the U.S. are cultivated rice and red rice. Wild rice is a different genus and is not sexually compatible with cultivated rice. As there is no commercial rice grown in Kansas and red rice only grows where there is cultivated rice, the possibility of outcrossing is effectively zero.
- To prevent intermingling of seeds with other crop plants, Ventria will use dedicated equipment, storage and processing facilities. The majority of the harvested seeds will be milled to flour and will not be shipped to any outside milling facilities.

¹ By convention, the gene is designated by small italic letters and the protein produced by that gene is designated by non-italicized letters, first letter capitalized.

- None of the introduced genes provide the engineered rice plants with any selective advantage over non-engineered rice in the ability to be disseminated or to become established in the environment.

The proposed field plantings are a controlled release of the regulated article into the environment. The protocols and field plot design as well as the procedures for termination of the field tests have been deemed sufficient to ensure that none of the engineered rice plants persist in the environment beyond the termination of the plantings. The proposed field tests do not present a significant impact on populations of non-target animal species, including any threatened or endangered species or critical habitat in Kansas. APHIS review and analyses of the data packages presented by the applicant indicate that the proposed field plantings do not present a risk of introduction and dissemination of a plant pest and should not have a significant impact, either individually or cumulatively, on the quality of the human environment.

II. Introduction

A. Intended uses for the developed rice lines

Ventria Bioscience (formerly Applied Phytologics) has been developing genetically engineered plants producing pharmaceutical and novel proteins since 1996. They intend to continue this work in 2007, growing several different rice lines containing three distinct novel proteins (described later in this Environmental Assessment), which will be extracted from rice seed.

Ventria intends to extract recombinant human lysozyme and lactoferrin from rice flour to be used as supplements in yogurts, meal replacement and performance beverages, bars (for example granola bars) and in nutritional supplement drinks. They also intend to use lysozyme and lactoferrin in the preparation of medical foods such as oral rehydration solutions. Ventria will also extract recombinant human serum albumin (HSA) protein to be used primarily for cell culture use.

B. Regulatory Authority

The authorities for regulation of genetically engineered rice are the Plant Protection Act of 2000, 7 U.S.C. 7701-7772, and USDA, APHIS regulations under 7 CFR § 340, "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which are Plant Pests or Which There is Reason to Believe are Plant Pests." A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxonomic groups listed in the regulation and is also a plant pest, or if there is a reason to believe it is a plant pest. In this submission, rice lines have been genetically engineered using techniques of microprojectile bombardment or disarmed *Agrobacterium*-mediated transformation using genes from at least one plant pest. The introduced DNA contains human, bacterial and synthetic gene sequences that have been engineered for optimal protein expression in rice. Regulatory and selectable antibiotic or

herbicide marker gene sequences from *E.coli*, *Agrobacterium tumefaciens*, rice, and/or *Streptomyces hygroscopicus* are also incorporated into these rice lines.

Generally, issuance of a permit for field trials of regulated articles is categorically excluded from requirements for an environmental assessment (EA) under APHIS NEPA implementing procedures (7 C.F.R. § 372.5(c)(3)(i)). However, when APHIS determines that a confined field release of genetically engineered organisms has the potential to significantly affect the quality of the human environment, as those terms are defined in 40 C.F.R. §§ 1508.27 and 1509.14, an environmental assessment or environmental impact statement will be prepared, pursuant to 7 C.F.R. § 372.5(d). This EA was prepared because the applicant intends to have plantings of these engineered plants in Geary County, Kansas for the next several years. The potential for cumulative impacts of these plantings in the same area raises new issues that this EA addresses. Future plantings are anticipated to increase in size and will be required to meet all the performance and mitigation measures described in the permit applications and all permit conditions.

III. Purpose and Need

A. Proposed Action

The proposed action is for APHIS, Biotechnology Regulatory Services (BRS), to issue permits for confined field plantings of several lines of rice that have been genetically engineered to express proteins for human lysozyme, lactoferrin or serum albumin. Lysozyme and lactoferrin lines contain a variety of regulatory gene sequences (i.e., promoters, terminators and enhancers) and selectable marker genes for hygromycin phosphotransferase (*hpt*). Serum albumin lines also contain similar regulatory gene sequences and may contain selectable marker genes for *hpt* and/or phosphinothricin acetyltransferase (*pat*).

The plantings will occur in at least 2 locations and individual rice lines will be physically isolated from each other to maintain product purity. This environmental assessment (EA) will evaluate the regulated articles in three permit applications: 06-278-01r, 06-278-02r and 06-285-02r.

B. Purpose

The purpose of this EA is to assess potential environmental effects resulting from confined field plantings of the engineered rice lines in Geary County, Kansas. Permit applications were submitted by Ventria Bioscience to USDA/APHIS/BRS on October 5 and October 12, 2006.

Identical or nearly identical rice lines have been the subject of previous Environmental Assessments by APHIS and portions of those assessments, when appropriate, will be incorporated in this EA or appendices by reference.

C. Need for This Action

Under APHIS regulations, the receipt of a permit application to introduce a genetically engineered organism requires a response from the Administrator:

Administrative action on applications. After receipt and review by APHIS of the application and the data submitted pursuant to paragraph (a) of this section, including any additional information requested by APHIS, a permit shall be granted or denied. 7 CFR 340.4(e)

IV. Alternatives to the Proposed Action

APHIS has considered the following three alternatives in response to the applicant's request for a permit:

Alternative 1:

No action/deny the permit applications. Under this alternative, field releases of the engineered rice plants would not be authorized.

Alternative 2:

Issue the permits for growing under the conditions proposed by the applicant. Under this alternative, field release of the engineered rice plants would be authorized at the specified locations with no additional conditions outside of those the applicant provided in the request. Standard permit conditions under 7 CFR § 340.4 would be required. Standard management practices, including use of some pesticidal and herbicidal sprays, will be included as part of the planting design.

Alternative 3:

Issue the permits with additional conditions for carrying out the field plantings. Under this alternative, field release of the engineered plants would be authorized. Supplemental permit conditions, based on APHIS analysis, the State of Kansas, and public comment on this environmental assessment, would be required. If warranted, based on environmental risk of escape of the engineered organism, APHIS will require mitigating measures to prevent spread of the organism outside the field production area. Standard management practices, including use of some pesticidal and herbicidal sprays, will be included as part of the planting design.

APHIS field test permits typically include supplemental permit conditions that may reflect input from the appropriate State regulatory officials. The supplemental permit conditions also specify requirements for additional post-planting and post-harvest volunteer monitoring reports. These post-planting and monitoring reports assist APHIS in evaluating specific field tests under permit and also provide guidance for evaluating

future proposed field tests. APHIS' proposed Supplemental Permit Conditions are described in Appendix 4. Several of these conditions are described:

- The plantings shall be at least ¼ mile from any commercial rice plantings.
- The applicant shall provide APHIS and State regulatory officials information on the location of the nearest commercial rice plants that are not part of the field test.
- The applicant shall provide APHIS and State regulatory officials a detailed map of the proposed plantings. One month after planting, the applicant will submit a detailed map of the planted sites.
- The applicant shall use screens on irrigation outlets to prevent movement of seeds/seedlings out of the field with water used to flood the field. They shall also employ flooding methods which create a closed system so that ungerminated seeds cannot leave the field site.
- A zone of 50 feet shall be maintained surrounding the field test site. A non-food or non-feed cover crop may be planted in this zone to prevent erosion or this area may remain fallow.
- In the subsequent growing season, the production sites and the 50 foot fallow zone may not be planted with rice unless similar transgenic rice is repeated. If the same crop does not follow in subsequent years, the site shall be monitored for volunteer rice plants at least 3 times during the following growing season. Any volunteer rice plants shall be destroyed before flowering.
- Ventria shall use equipment dedicated to these plantings as outlined in their SOPs. This equipment shall not be used for any other purposes during the course of the field test. After the field test is completed, all equipment shall be thoroughly cleaned and inspected to ensure that all genetically engineered seed and other plant material has been removed and destroyed.
- Ventria shall scout for red rice during the entire growing season both within the growing plots and for ¼ mile from the production fields. Any red rice found shall be destroyed and shall not be allowed to flower. Ventria shall also inform APHIS if any red rice is found within the ¼ mile zone or in their production plots.

Ventria is also required to maintain at least 1320 feet isolation from other rice. The Association of Official Seed Certifying Agencies (AOSCA) standards for foundation certified rice seed require at least 10 feet from other rice. In practice, there is no known commercial rice grown in the state of Kansas.

V. Description of the Field Plantings/ Affected Environment

Purpose

The purpose of this proposed introduction is multi-fold:

- For continued testing of gene stability over several generations of seed production,
- For further molecular and protein testing,
- To develop seed stock for product development and breeding, and
- For scale-up.

The regulated introduction is proposed for planting between March and May 2007 and would grow in the field until harvest (estimated September-November 2007). Ventria has proposed to plant acreages comparable in size and scope to those grown in North Carolina in 2006 (http://www.aphis.usda.gov/brs/ph_permits.html). See permit numbers 05-293-01r, 05-332-01r, and 05-332-02r). One noted change is the addition of rice lines developed to produce human serum albumin.

APHIS has reviewed and approved protocols that are proposed by Ventria to prevent the escape and dissemination of these plants, submitted on APHIS Form 2000. In addition, Standard Operating Procedures (SOPs) submitted by Ventria identify more detailed instructions and provide additional guidance.

Field Plot Design, Breeding Procedures and Agricultural Practices

Plot Design and Location

Ventria's plantings are proposed in Geary County, Kansas. There are no known commercial rice fields in Geary County or neighboring counties. The area has not had a history of rice farming; soybean, corn and winter wheat have been grown in the area for many years. Ventria will monitor for commercial rice production and scout for weedy red rice within ¼ mile of its plantings.

Rice is highly self-pollinating and is not generally pollinated by insects. AOSCA-certified seed regulations for foundation rice seed require a minimum isolation distance from other rice varieties of at least ten feet when hand- or machine-planted. A 50 foot fallow zone and a separation distance of ¼ mile from any other rice (one hundred thirty two times the AOSCA standard) as proposed by the applicant should be more than adequate to prevent unintended release of the transgenic rice into adjacent fields. To prevent erosion, the fallow zone may be planted with a low-growing crop that will not be used for food or feed. The rice will be grown in flooded/water soaked fields.

Agricultural Practices

The rice will be allowed to self pollinate to produce seed. No breeding operations are planned under this permit. Agricultural practices consistent with growing healthy rice plants will be used. Weeds will be controlled by herbicide applications. If necessary, pesticides such as insecticides and/or fungicides will be used to control insect pests and disease. Any pesticides used will be applied by appropriately trained personnel. If necessary, EPA-registered pesticides will be used to control insect pests. Fields will be monitored for noxious weeds and other plant pests during the growing season. Three times during the growing season the plants will be inspected for traits such as weediness, resistance/susceptibility to insects or disease, or unusual differences in plant growth or morphology. The plot will be inspected weekly while personnel also conduct agricultural practices. Nearby fields will likely have crops such as corn, soybean and winter wheat.

Field Observation and Monitoring

The applicant has thoroughly described field site monitoring and management practices that should provide the necessary degree of biological and physical confinement.

Confinement practices and permit conditions are described in section IV Alternative 3 listed above. Additionally:

- there are no known fields of commercial rice in Geary or surrounding counties in Kansas.
- there are no known populations of red rice in Kansas.

Alteration in susceptibility to disease or insects

Since Ventria first began planting these rice lines, they have not noted increased susceptibility to disease or insect pests. There has been no intentional genetic change in these plants to affect their susceptibility to disease or insect damage. Neither the selectable marker genes, *hpt* and *pat*, nor the lysozyme, lactoferrin or *hsa* genes are expected to alter the susceptibility of the transgenic rice plants to disease or insect damage. Execution of the prescribed periodic monitoring of the field plots will allow the detection of any unexpected infestation by plant disease organisms or animal pests. Ventria is required to report any such unanticipated effects to APHIS.

Termination of the field test and final disposition of the test plants

Seed will be machine-harvested using a dedicated combine and dried and cleaned in a designated/dedicated staging area in the same county using a dedicated dryer and cleaner. The seeds will be stored in dedicated storage bins until processed. During the process, the seed will be dehusked using a dedicated dehusker and will be milled in a dedicated staging area using a dedicated mill. Milled rice flour will be shipped to designated locations for subsequent processing. Any devitalized waste material from the milling operation will be returned to the field test site and incorporated into the soil or otherwise properly disposed of by an alternate, APHIS-approved method. All the operations up to milling will be performed in an APHIS-inspected dedicated area using dedicated equipment. Most material will be shipped only after milling. Some viable seed may be stored in APHIS-inspected locations and/or shipped to other locations for analysis or planting in subsequent seasons. All interstate shipments will be done under APHIS permits.

After harvest, as soon as the weather allows, Ventria will burn and disk the fields to encourage rapid decomposition of all plant and seed materials that may remain. Off-season irrigation may also be used to accelerate the germination of any remaining viable seed prior to winter. Ventria plans to grow these rice varieties in the same locations in subsequent years. However, if a change to a different crop is anticipated, the field will be fallowed for one cropping season after the harvest of the transgenic lines. During this 18 month period (the period between harvest year 1 to planting year 3), the field will be monitored at least three times in the following growing season and managed to induce germination and/or decomposition of any viable seed. Ventria employs a “pureland”

procedure to control volunteers. This includes flushing the field with water during the growing season to induce germination of weed and rice seed, drying the field to kill seedlings, and then disking to dry and kill seedlings. This process is repeated three times.

Security of the planting sites

The planting sites are expected to provide adequate physical security. The contract farmers own the fields to be planted with the transgenic rice. The surrounding fields outside the food/feed crop fallow zone will be planted to soybean, corn or winter wheat. The sites are not prone to flooding. The closest body of water is the Smokey Hill River which is located approximately 1 mile to the south of one proposed plot and approximately 1 mile west of another proposed plot. In both locations, the river is separated from fields by a steep embankment and these two particular locations do not have a history of flooding. The Kansas River is located about 3-4 miles from the proposed planting sites. If other sites in Geary County are proposed prior to issuance of these permits and subsequent planting, APHIS will assess them to determine whether they may also be appropriate for these plantings.

VI. Potential Environmental Impacts

Other than location-specific references, descriptions of the potential environmental impacts to Alternatives 1, 2 and 3 are incorporated here by reference to Environmental Assessments (EA) completed for permits 05-117-01r (lactoferrin, Section VIII, pp 16-24), 05-117-02r (lysozyme, Section VIII, pp 16-24) and 96-355-01r (human serum albumin, Section V, pp 5-7). These documents can be found at the following links: http://www.aphis.usda.gov/brs/ph_permits.html and <http://www.isb.vt.edu/cfdocs/fieldtests3.cfm>. Issues addressed included the potential for persistence in the environment, the potential for gene transfer, potential impacts from use of the marker genes, potential impact on native floral and faunal communities, potential alteration in susceptibility to disease or insects, potential impacts on existing agricultural practices, potential impact on adjacent row crops, fate of transgenic DNA, potential impacts on human health, potential cumulative environmental effects and special considerations regarding other environmental statutes. A discussion of potential effects of the plantings on Threatened and Endangered species (TES) and critical habitat specific to Kansas follows.

Effects of field plantings on Threatened and Endangered Species

The proposed field plantings are confined releases of the regulated articles into the environment in Geary County, Kansas. The proposed planting sites are within 10 miles of Ventria's storage and processing facility in Junction City, KS. Some sites may be within ½ mile of Dickinson County to the west. Lists of TES and proposed TES, as well as an analysis of designated critical habitat and proposed designated habitat, were obtained from the U.S. Fish and Wildlife Service (USFWS). Upon review of the information obtained from USFWS, it was determined that there are seventeen TES in the state and

no critical habitat in the proposed planting areas. These documents can be found at the following links:

http://ecos.fws.gov/tess_public/StateListing.do?state=KS&status=listed and <http://crithab.fws.gov/>

The TES listed include the Indiana bat (*Myotis sodalis*), gray bat (*Myotis grisescens*), American burying beetle (*Nicrophorus americanus*), gray wolf (*Canis lupus*); 6 bird species: whooping crane (*Grus americana*), Eskimo curlew (*Numenius borealis*), bald eagle (*Haliaeetus leucocephalus*), Least tern (*Sterna antillarum*), black-capped Vireo (*Vireo atricapilla*), piping plover (*Charadrius melodus*); 4 fish species: Neosho madtom (*Noturus placidus*), Pallid sturgeon (*Scaphirhynchus albus*), Arkansas River shiner (*Notropis girardi*), Topeka shiner (*Notropis topeka*); and three endangered plants: running buffalo clover (*Trifolium stolonifera*), Mead's milkweed (*Asclepias meadii*) and the western prairie fringed orchid (*Platanthera praeclara*) as existing or once existing in Kansas. Only 2 of these species have been recorded as identified in Geary County: the Topeka shiner and the Least tern. Neither of these would likely be exposed to or consume material from these plantings. The Topeka shiner is a small minnow, occurring typically in quiet, clear, open pools of river headwaters and creeks. It primarily consumes midge larvae and aquatic invertebrates. The Least tern is considered a shore bird and primarily consumes insects and fish. Several of the other species listed are presumed absent from Kansas. Given the non-toxic nature of the rice and proteins produced, other species, even if exposed, would not be expected to be affected.

BRS has reviewed this data in accordance with a process mutually agreed upon with the U.S. Fish and Wildlife Service (USFWS) to determine when a consultation, as required under Section 7 of the Endangered Species Act, is needed. APHIS has reached a determination that release under these permits (06-278-01r, 06-278-02r and 06-285-02r) would have no effect on designated critical habitat or listed threatened or endangered species and consequently, a written concurrence or formal consultation with the USFWS is not required for this EA.

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Appendix 1: Description of Rice Biology

A description of rice biology is incorporated here by reference to the Environmental Assessment (EA) completed for permit 05-117-01r (Section V, pp 7-9). This document is applicable to all three permit applications that are the subject of this EA and can be found here: http://www.aphis.usda.gov/brs/ph_permits.html. Topics covered in the referenced Environmental Assessment discuss rice systematics, genetic improvement, weediness, modes of gene escape, outcrossing, movement of seed by animals, movement of seed by water and movement by human error.

Appendix 2: Description of the Regulated Rice Plants

Ventria has engineered these rice plants to produce human lysozyme, lactoferrin or human serum albumin in the seeds. The engineered rice (*Oryza sativa* ssp *japonica* var Taipei 309, M202 or M103) varieties are medium grain, Japonica types. They are not grown widely in the U.S. In each case, gene expression is targeted to the developing seed so the different proteins are not produced in other parts of the plant.

Detailed descriptions of the lactoferrin and lysozyme producing rice plants are incorporated here by reference to Environmental Assessments (EA) completed for permits 05-117-01r (lactoferrin, Section VI, pp 9-13) and 05-117-02r (lysozyme, Section VI, pp 9-13). These documents can be found here: http://www.aphis.usda.gov/brs/ph_permits.html. Topics covered in the referenced EAs discussed gene vectors, selectable markers, the genes of interest, characterization of the engineered plants, molecular characterization, protein characterization, assessments of protein toxicity and allergenicity, thermal and gastric stability and potential environmental exposure levels.

Ventria has made minor changes to its gene constructs and method of transformation for its HSA-containing rice lines. The plants developed for current use (permit application 06-278-02r) were generated using *Agrobacterium*-mediated transformation and the use of two selectable marker genes. The marker genes hygromycin phosphotransferase and phosphinothricin acetyltransferase (*hpt* and *pat*) are included to allow selection of transgenic tissues in the laboratory using the antibiotic hygromycin and/or the herbicide bialaphos. Neither selectable marker gene is expressed in mature rice tissues. In many years of testing, these rice plants have not exhibited plant pest characteristics when modified by the introduction of these genes and their associated regulatory sequences. These sequences do not have any inherent plant pest characteristics and are not known to enhance gene transfer from plants to other organisms.

Brief descriptions of the proteins to be produced in the engineered rice plants are included here.

Lysozyme is ubiquitous in the human body where it acts as a protective barrier against environmental agents and, in doing so, helps prevent infection. Lysozyme is a small enzyme that attacks the protective cell walls of bacteria. It breaks the carbohydrate

chains in bacterial cell walls, destroys the structural integrity, and causes the bacteria to burst under their own internal pressure. Lysozyme plays a role in antibacterial disease defense, particularly against gram-positive bacteria. Both antiviral (O'Neil et al., 2001) and antifungal (Samaranayake et al., 2001) activity has been reported. Lysozyme in cattle plays a role in gastric digestion and in chicken egg whites functions as an antibiotic. Lysozyme occurs in tears, nasal mucus, milk, saliva, blood serum, many types of tissues and secretions of different animals, including vertebrates and invertebrates, and in plant latex (O'Neil et al., 2001).

Lactoferrin is an iron-binding glycoprotein (~80 kDa) consisting of approximately 700 amino acids and is found in milk, tears, saliva and other mammalian body secretions (Ekstrand, 1994). In human milk, it is a major component of whey protein with 6-8 grams/liter in colostrum (foremilk) and 2-4 grams/liter in "mature" milk (Harper, accessed 2006). Bovine (cow) milk contains 5-20 times less by weight. Lactoferrin is reported to have broad antimicrobial properties (against bacteria, fungi, and viruses), an immune system regulatory function, anti-inflammatory properties, antioxidant activity, toxin binding properties and anti-cancer activity (Harper, accessed 2006). Lactoferrin may exist in several forms: iron deficient (apo-form), iron sufficient (holo-form), and an "activated" form (Naidu, 2002). The form it takes depends primarily upon pH, the citrate/bicarbonate ion ratios, and the iron content of the medium. Treatment of lactoferrin under specific chemical conditions results in "activation" of lactoferrin. The iron deficient (apo-) and activated forms, due primarily to their ability to sequester iron, are most active in their antimicrobial properties (Naidu, 2002).

Human serum albumin (HSA) is a soluble, monomeric protein which comprises about one-half of the blood serum protein. The protein is encoded by the *alb* gene and is produced in the liver. It functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones and plays a role in stabilizing extracellular fluid volume. It is used in medical practice to replace blood volume in burn victims, patients suffering acute traumatic shock, and those undergoing certain types of surgery. It has no reported oral or dermal activities.

References

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Antifungal effects of lysozyme and lactoferrin against genetically similar
sequential *Candida albicans* isolates from a human immunodeficiency virus-
infected southern Chinese cohort. J. Clin. Microbiol. **39**: 3296-3302.

Appendix 3: Threatened or Endangered Species for Kansas (17 species) (http://ecos.fws.gov/tess_public/StateListingAndOccurrence.do?state=KS)

Animals

- Bat, gray (*Myotis grisescens*)
- Bat, Indiana (*Myotis sodalis*)
- Beetle, American burying (*Nicrophorus americanus*)
- Crane, whooping (*Grus americana*)
- Curlew, Eskimo (*Numenius borealis*) (shorebird)
- Eagle, bald (*Haliaeetus leucocephalus*)
- Madtom, Neosho (*Noturus placidus*)(fish)
- Plover, piping (*Charadrius melodus*)(shorebird)
- Shiner, Arkansas (*Notropis girardi*)(fish)
- Shiner, Topeka (*Notropis topeka* (=tristis))
- Sturgeon, pallid (*Scaphirhynchus albus*)
- Tern, least (*Sterna antillarum*)
- Vireo, black-capped (*Vireo atricapilla*)
- Wolf, gray (*Canis lupus*)

Plants

- Clover, running buffalo (*Trifolium stoloniferum*)
- Milkweed, Mead's (*Asclepias meadii*)
- Orchid, western prairie fringed (*Platanthera praeclara*)

Appendix 4: APHIS' Proposed Supplemental Permit Conditions

I. Compliance with Regulations

1. Any regulated article introduced not in compliance with the requirements of 7 Code of Federal Regulation Part 340 or any standard or supplemental permit conditions, shall be subject to the immediate application of such remedial measures or safeguards as an inspector determines necessary, to prevent the introduction of such plant pests. The responsible party may be subject to fines or penalties as authorized by the Plant Protection Act (7 U.S.C. 7701-7772).
2. This Permit (APHIS form 2000) does not eliminate the permittee's legal responsibility to obtain all necessary Federal and State approvals, including: (A) for the use of any non-genetically engineered plant pest or pathogens as challenge inoculum; (B) plants, plant parts or seeds which are under existing Federal or State quarantine or restricted use; (C) experimental use of unregistered chemicals; and (D) food, feed, pharmacological, biologic, or industrial use of regulated articles or their products and co-mingled plant material. In the latter case, depending on the use, reviews by APHIS, the U.S. Food and Drug Administration, or the U.S. Environmental Protection Agency may be necessary.
3. The procedures, processes, and safeguards used to prevent escape, dissemination, and persistence of the regulated article as described in the permit application, in APHIS-approved Standard Operating Procedures (SOPs) and, in the supplemental permit conditions must be strictly followed. The permittee must maintain records sufficient to verify compliance with these procedures, including information regarding who performed the activity. Persons performing such activities shall have received training as described in a training program submitted to and approved by APHIS. These records are subject to examination by APHIS. APHIS must be notified of any proposed changes to the protocol referenced in the permit application.

II. Reporting Unauthorized Releases and Unintended Effects

1. According to the regulation in 7 CFR § 340.4(f)(10)(i), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article.
 - For immediate oral notification, contact APHIS/BRS Compliance Staff at (301) 734-5690 and ask to speak to a Compliance and Inspection staff member.
 - In the event of an emergency and you are unable to reach APHIS/BRS Compliance Staff at the above number, you may call:

The APHIS/BRS Regional Biotechnology Coordinator assigned to the state, where the field test occurs

For Western Region, contact Ralph Stoaks by phone at (970) 494-7573 or e-mail Ralph.D.Stoaks@aphis.usda.gov

Or

The APHIS/PPQ Regional Biotechnology Coordinator assigned to the state where the field test occurs

For Western Region, contact Stacy Scott by phone at 970-494-7577 or e-mail Stacy.E.Scott@aphis.usda.gov

Or

The APHIS State Plant Health Director for the state where the field tests occur.

Mr. Russell McKinney

Phone: 785-270-1381

Fax: 785-235-1464

Russell.A.McKinney@aphis.usda.gov

2. According to the regulation in 7 CFR § 340.4(f)(10)(ii), APHIS shall be notified in writing as soon as possible but within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the permit application or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).
3. Written notification should be sent by one of the following means:

By e-mail:

BRSCCompliance@aphis.usda.gov

By mail:

Biotechnology Regulatory Services (BRS)

Compliance and Inspection Branch

USDA/APHIS

4700 River Rd. Unit 147

Riverdale, MD 20737

III. Perimeter Fallow Zone

1. To ensure that transgenic plants are not inadvertently commingled with plants to be used for food or feed, a perimeter fallow zone of at least 50 ft. must be maintained around the transgenic test site in which no crops are grown to be harvested or used for food or feed.
2. The perimeter fallow zone shall be managed in a way that allows detection and destruction of volunteer plants that are the same as, or sexually compatible with, the transgenic plants.

IV. Dedicated Planting and Harvesting

1. To ensure that the regulated article is not inadvertently removed from the site, planting and harvesting equipment must be dedicated for use in the permitted test site(s) from the time of planting through the end of harvest.
2. After harvest, you will not be required to obtain APHIS authorization to use this equipment on APHIS -permitted sites (same sites or different sites) planted with same transgenic crop, with the target protein(s) authorized under this permit, in subsequent growing seasons under an extension of this permit or a different permit.
3. Authorization is required from APHIS before this planting and harvesting equipment can be used on sites planted to crops not included under this permit. Ventria must notify APHIS/BRS and the State Regulatory Official at least 21 calendar days in advance of cleaning this equipment for this purpose so that APHIS may schedule an inspection to ensure that the equipment has been cleaned appropriately.

V. Cleaning of Equipment

1. To minimize the risk of seed movement and commingling, equipment used for planting and harvesting, as well as other field equipment (e.g. tractors and tillage attachments, such as disks, plows, harrows, and subsoilers) used at any time from the time of planting through the post-harvest monitoring period must be cleaned in accordance with procedures submitted to and approved by APHIS before they are moved off of the test site.
2. Equipment used to transport seeds or harvested material must be cleaned prior to loading and after transportation to the authorized site in accordance with procedures submitted to and approved by APHIS.

3. Seed cleaning and drying must be performed in accordance with the procedures submitted to and approved by APHIS to confine the plant material and minimize the risk of seed loss, spillage, or commingling.

VI. Use of Dedicated Storage Facilities

1. Dedicated facilities (locked or secured buildings, bins, or areas, posted as restricted to authorized personnel only) must be used for storage of equipment and regulated articles for the duration of the field test.
2. Before returning these facilities to general use, they must be cleaned in accordance with procedures submitted to and approved by APHIS. **Ventria must notify** APHIS/BRS and the State Regulatory Official at least 21 calendar days in advance to allow for APHIS to schedule an inspection to ensure that the facilities have been cleaned appropriately. APHIS authorization should be received before facilities are returned to general use.

VII. Post Harvest Monitoring

The field test site including the perimeter fallow zone must be monitored for the presence of volunteer rice plants for 1 year after termination of the field test. Viable plant material should not remain at the test site following termination.

VIII. Post Harvest Land Use Restrictions

1. Production of food and feed crops at the field test site and the perimeter fallow zone is restricted during the growing season that follows harvest or termination of the field test.
2. Permission must be obtained from APHIS/BRS prior to planting any food or feed crop at the field test site and perimeter fallow zone during the post-harvest monitoring period. Requests for such permission are not encouraged and will not be granted in cases where there is a reasonable potential for plant material derived from, or originating from, the regulated articles to become mixed with the proposed food or feed crop during harvesting.

IX. Inspections

1. APHIS Biotechnology Regulatory Services (BRS) and/or an APHIS/PPQ Regional Biotechnologist, APHIS/BRS Regional Biotechnology Coordinator or APHIS State Plant Health Director may conduct inspections of the test site, facilities, and/or records at any time.
2. APHIS may invite the FDA or State Regulatory Officials to participate in these inspections.
3. Inspections will likely correspond to the beginning of the field test, mid-season or during flowering, at and/or following harvest, and during the post-harvest monitoring period.
4. Inspections will include examination of records that verify compliance with regulations and SOPs.

X. Reports and Notices

Send notices and all reports (CBI and CBI-deleted or non-CBI copies) to BRS by e-mail, mail, or fax.

BRS E-mail:

BRSCompliance@aphis.usda.gov

BRS Mail:

Biotechnology Regulatory Services (BRS)
Compliance and Inspection Branch
USDA/APHIS
4700 River Rd. Unit 147
Riverdale, MD 20737

BRS Fax:

Compliance and Inspection Branch
(301) 734-8669

In addition, fax the CBI deleted or non CBI version of the pre-planting and pre-harvest (termination) notices to the State Regulatory Official(s)

Contact information for State Officials

<http://www.nationalplantboard.org/member/index.html>

1. Pre-Planting Notice

At least 7 calendar days before planting, submit a Pre-Planting notice that includes the following information for each field test site:

- i. Provide APHIS/BRS with the contact information for each field test site.
- ii. Indicate if planting and harvesting equipment will be moved between authorized field test sites.
- iii. A map that clearly identifies the site location to facilitate any inspections by USDA personnel.
- iv. The planned number of acres for each gene construct.
- v. The planned planting date

2. Planting Report

Within 28 calendar days after planting, submit a planting report that includes the following information for each field test site:

- i. A map of the site, with sufficient information to locate it, that includes: the state, county, address, GPS coordinates for each corner of the plot;
- ii. The location and the approximate number and/or acres of transgenic plants which were actually planted at the test site for each of the target proteins;
- iii. The total acreage of the test plot (exclude border rows, if any);
- iv. The distance from the genetically engineered plants to the nearest plants of the same crop which will be used for food, feed, or seed production. A survey should be done within at least ¼ mile of the planting sites.
- v. The actual planting date.

3. Pre-Harvest/ Termination Notice

At least 21 calendar days prior to the anticipated harvest or termination, submit a Notice indicating the planned date of harvest **or** termination and the contact information for each field test site. For multiple harvests, submit the notice prior to the initial harvest.

4. Field Test Report

Within 6 months after the end of the field test (final harvest or crop destruct), the permittee is required to submit a field test report. Field test reports shall include:

- i. APHIS reference number
- ii. Methods of observation.
- iii. Resulting data.
- iv. Analysis of all deleterious effects on plants, non-target organisms, or the environment.
- v. A list of the lines planted at each site
- vi. Disposition table

The disposition table should contain the following information: site name (or GPS), crop, gene, harvest date, and disposition of harvested material.

The disposition table is a formal record of how the regulated material was removed from the environment. An accounting of the harvested material should be provided with regards to what material is harvested, how much material is harvested per site, what is done to devitalize residual and harvested

material at the site, where the harvested material is transported, stored and further processed up to the time it is taken to a contained facility.

5. Monitoring Report

Within 3 months after the end of the monitoring period, submit a volunteer monitoring report. The report must include:

- i. Dates when the field site and perimeter fallow zone were inspected for volunteers.
- ii. Number of volunteers observed.
- iii. Any actions taken to remove or destroy volunteers.

XI. Additional Data Requirements

No additional requirements.